Current Biology, Vol. 13, R652–R654, August 19, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/S0960-9822(03)00574-8

Ribosomal Translocation: Sparsomycin Pushes the Button

Dispatch

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New insights into the way that the tRNA:mRNA complex is translocated on the ribosome during protein synthesis have come from the recent observation that binding of the antibiotic sparsomycin in the peptidyl transferase center can trigger these large-scale movements.

Following the formation of every peptide bond during protein synthesis, the tRNA:mRNA complex must be translocated by precisely three nucleotides through the densely packed interior of the ribosome. While recent crystal structures have revealed a detailed molecular view of an RNA-only active site for peptide bond formation, far less is known about how two 25 kDa tRNA substrates are coordinately moved more than 20 Å through the meshwork of RNA and protein interactions that form the functional core of the ribosome.

Fredrick and Noller [1] have now provided stunning evidence that long-range communication networks operate during translocation by showing that binding of the antibiotic sparsomycin to the peptidyl transferase center of the large subunit of the ribosome triggers translocation of the tRNA:mRNA complex on the small subunit, an event that normally requires elongation factor G (EF-G) and GTP hydrolysis. Although it is unclear how binding of a small molecule in the active site can trigger movements more than 70 Å away, where the mRNA and tRNA anticodon interact, the results provide a new and intriguing perspective for understanding the dynamic process of translocation. In circumventing the requirement for EF-G and the added energy of GTP hydrolysis, sparsomycin apparently influences ribosomal structure as if it were directly pushing a button that is normally accessed via signal transduction from EF-G on the exterior of the ribosome.

During the elongation cycle of protein synthesis, the tRNA substrates occupy a series of discrete binding sites spanning both ribosomal subunits, the A (aminoacyl), P (peptidyl) and E (exit) sites (Figure 1). As a result of peptide bond formation, two new tRNA species are formed on the ribosome, a deacylated tRNA (the donor species that previously carried the peptidyl chain) and a peptidyl tRNA (the acceptor species that acquired the peptidyl chain) which must be translocated to open up the A site for the next round of elongation.

While there is universal agreement about the existence of the three distinct tRNA binding sites, how the tRNAs move (translocate) from one site to the next following peptide bond formation, and whether they occupy intermediate binding states during the translocation process is the subject of considerable debate. mRNA primer extension experiments ('toeprinting') have unambiguously shown that movement of the tRNA:mRNA complex with respect to the small subunit of the ribosome does not normally occur in the absence of EF-G and GTP. Chemical footprinting and fluorescence experiments, however, have suggested that movement of the acceptor ends of the tRNAs with respect to the large ribosomal subunit occurs immediately following peptide bond formation, even without EF-G function — this proposed intermediate state of tRNA binding is known as the 'hybrid state' [2,3]. While cryoelectron microscopic reconstructions of the ribosome have seen tentative views of hybrid bound tRNAs [4], most structural data thus far has been unable to confirm that the hybrid state of tRNA binding is normally populated during translation.

How are the large-scale movements of the tRNA:mRNA complex promoted by EF-G? EF-G is thought initially to interact with the flexible and exposed stalk region of the ribosome. This interaction promotes rapid GTP hydrolysis, leading to conformational changes that ultimately allow the extended domain IV of EF-G to interact productively with the decoding region of the small subunit, perhaps mimicking tRNA, biasing movement of the tRNA:mRNA complex in the forward direction. Interestingly, there is no evidence that EF-G interacts directly with the peptidyl transferase center of the large ribosomal subunit during translocation.

Although one might predict that regions proximal to the mRNA:tRNA complex in the small subunit would have the greatest effect on mRNA:tRNA movement, there is growing evidence that the acceptor ends of the tRNAs can greatly influence the energetics and accuracy of translocation. Mutagenesis experiments have shown that molecular contacts between the CCA-end of the initially P site bound tRNA and the E site of the ribosome are critical for translocation [5–7]. Similarly, the composition of the acceptor end of the initially A site tRNA is critical in specifying both rapid and accurate translocation. Translocation is relatively slow when deacylated tRNA fills the pre-translocation A site, but its rate increases as more authentic peptidyl-like moieties are appended to this tRNA; with a dipeptidyl tRNA, fMet–Phe–tRNA^{Phe}, translocation proceeds rapidly at about 20 sec^{-1} [8,9].

Other recent experiments, using a primer extension toeprinting assay to monitor the accuracy of translocation, found that the presence of a peptidyl moiety on the acceptor tRNA was again critically important [10]. When EF-G is added to pre-translocation state ribosomes, three-nucleotide, 5′ to 3′ movement occurs far more consistently when *N*-acetylated aminoacylated tRNA is provided as the peptide bearing tRNA in the A site. Strikingly, when deacylated or aminoacylated tRNA (Phe–tRNA^{Phe}) is used instead, only a small fraction of the ribosomes translocate

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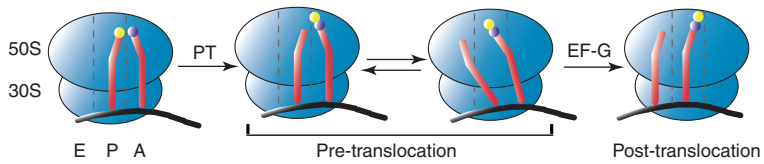

Figure 1. Movements of the tRNA:mRNA complex on the ribosome during the translation elongation cycle.

accurately — the remainder of the complexes move when EF-G is added, but aberrantly, to nearby cognate codons. Thus, movement of the mRNA and tRNA are uncoupled when appropriate contacts with the acceptor end of the tRNA are not established in the large subunit of the ribosome. How then is this information communicated to the small subunit where the gross movements of the tRNA:mRNA complex take place?

In their most recent work, Fredrick and Noller [1] provide another vital clue by showing that binding of the small molecule antibiotic sparsomycin in the active site of the large subunit of the ribosome can actually trigger translocation in the absence of EF-G and the energy of GTP hydrolysis. The translocation is accurate and relatively fast. While three to four orders of magnitude slower than the EF-G catalyzed event, the rate (0.005 sec^{-1}) is 900-fold faster than the observed background rate and considerably faster than spontaneous translocation rates previously seen in systems where the ribosomes have been chemically modified to increase translocation [11,12]. Little translocation is observed using deacylated tRNAs, likely because the peptidyl moiety contributes to sparsomycin binding. Finally, the sparsomycin-catalyzed event is sensitive to the translocation inhibitor viomycin but not to thiostrepton. As thiostrepton manifests its effects through interactions with the GTPase activating domain on the exterior of the ribosome, sparsomycin must act downstream from these earliest events in EF-G's signal transduction pathway.

What needs to be rationalized is how the small change in free energy from sparsomycin binding can be productively used to drive the large-scale movements of the tRNA:mRNA complex on the dynamic ribosome. Structural and biochemical approaches indicate that sparsomycin stabilizes P site tRNA interactions in the ribosome and effectively blocks A site substrate binding (Figure 2A) [13,14]. In one recent structure [14], sparsomycin is packed along the backbone of C75 and A76 of the P site tRNA and its peptide, and the uracil-like moiety of sparsomycin stacks directly on the universally conserved nucleobase A2602. The most conspicuous difference between the model pre-translocation ribosome structure and the sparsomycin bound structure is a simple rotation of the nucleobase of A2602 around its glycosidic bond (Figure 2A,B).

From such views, Fredrick and Noller [1] propose that the presence of sparsomycin might mimic interactions normally made by U2584 and U2585 (when EF-G is involved) leading to movement in A2602 and consequent alterations in the energetics of translocation. Perhaps the movement of A2602 is what allows translocation to happen — the 'button' — though it remains unclear how EF-G might induce such a structural rearrangement of A2602 in the active site. Such a role for A2602 is, however, consistent with its early identification as a key nucleotide related to tRNA movement [3] and the interpretation of recent structural data [15]. Similar conformational changes have been observed in the small ribosomal subunit at the universally conserved G530 which rotates around its glycosidic bond in response to cognate pairing interactions in the decoding site [16].

What insights into the movements of tRNA substrates on the ribosome can be gleaned from the observation of sparsomycin-promoted translocation? The hybrid states model [3] proposes that tRNAs move in a stepwise fashion on the ribosome, thus providing an intermediate state *en route* to the final more stable state following mRNA:tRNA translocation. This intermediate state of tRNA binding may well be the one that is recognized by EF-G as it is transiently sampled. By binding to the A site, sparsomycin may affect the energetics of the reaction in multiple ways. Ground state destabilization of the pre-hybrid state would be an obvious means to prevent backward sampling — and thus promote forward motion along the reaction pathway (Figure 2C). Based on what we see in the structure, sparsomycin seems likely to act in this way by sterically hindering access to the A site (Figure 2D). Further, the striking conformational changes seen in the active site at A2602 may stabilize the hybrid ground state or the transition state for progression from the hybrid to the post-translocation state. Some of the difficulties encountered in isolating and characterizing the hybrid state of tRNA binding on the ribosome may be reconciled by the view that this state, while integral to translocation, may be metastable and thus difficult to directly observe. Biochemical experiments that have followed the reactivity of pre-translocation state ribosomes with puromycin have led to similar conclusions [17] (our unpublished observations).

The unexpected observation that sparsomycin can promote translocation reminds us that the earliest ribosomes must not have depended on GTPases for function. Protein synthesis, or rather polymerization, must initially have been driven using primarily the energy of bond formation to promote directional synthesis, relying both on the dynamic properties of the ribosomal RNA and on the flexibility of tRNA to proceed through a cycle with surmountable energy barriers. Ribosomal proteins improved the accuracy and processivity of translation, but slowed it down by increasing the energy of the transition states, and so ultimately conscripted the energy of GTP hydrolysis for translocation. Such a view is supported by early experiments of Spirin and colleagues [18], and some more recent experiments [19], showing that when certain ribosomal proteins are removed from

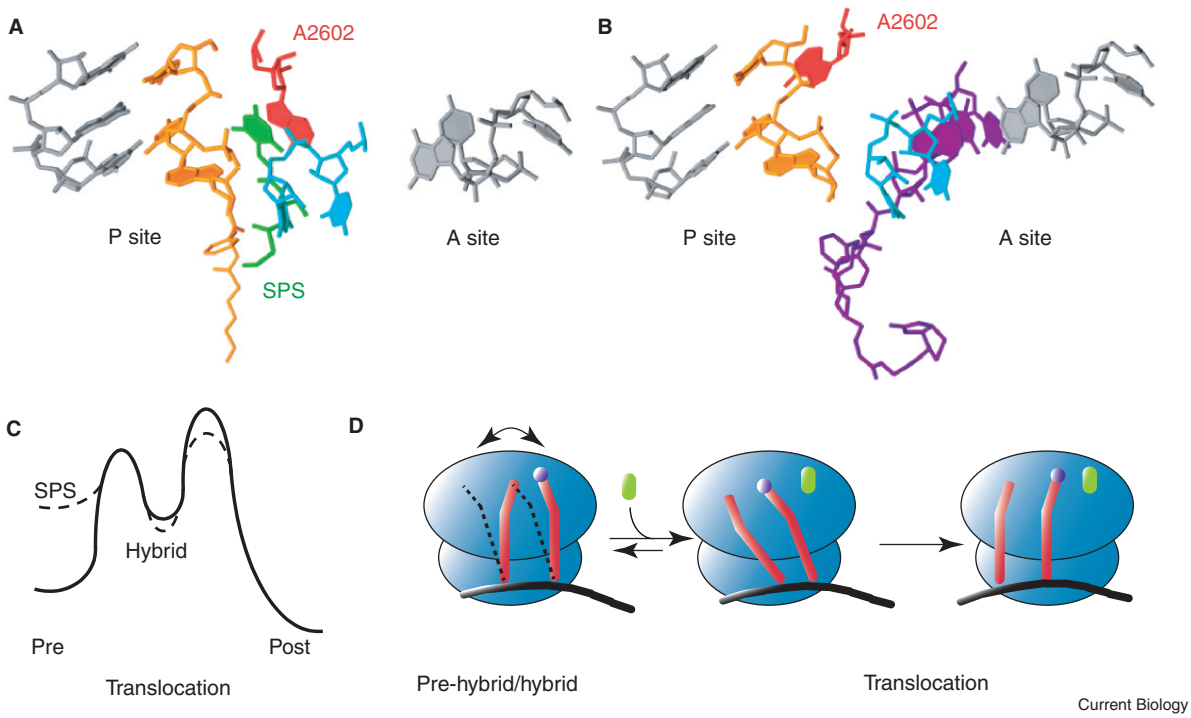

Figure 2. A model for sparsomycin-promoted translocation.

(A) Structure of active site with peptidyl tRNA analog (CCA-Phe-caproic-acid-biotin; orange) bound in P site along with sparsomycin (SPS, green) [14]. A and P loops (grey), A2602 (red) and U2584 and U2585 (blue). (B) 'Pre-translocation' active site structure with CCA in P site (orange) and peptidyl tRNA analog in A site (purple) [20]. (C) An energy diagram showing possible changes in free energy during translocation and how sparsomycin (dotted line) might affect these changes. (D) Sparsomycin-catalyzed translocation depicted as a stabilization of the 'hybrid' state of tRNA binding.

the ribosome, spontaneous translocation rates increase. When reduced to its most fundamental level, translation is likely, after all, to be RNA-based. A functionally relevant and simple conformational switch that is induced by small molecule binding declares that one is examining the very heart of ribosome function. We hope that more clues of this nature will be forthcoming.

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